Vertical and longitudinal distribution patterns of different bacterioplankton populations in a canyon-shaped, deep prealpine lake

Salcher, Michaela M ; Pernthaler, Jakob ; Frater, Nakita ; Posch, Thomas

Abstract: The pelagic zone of large, deep freshwater lakes features pronounced horizontal and vertical gradients of physicochemical parameters, which in turn might allow for a nonuniform occurrence of specifically adapted bacterial taxa. We, therefore, studied the spatial distribution patterns of different heterotrophic bacteria, picocyanobacteria, and the dominant primary producer, the filamentous cyanobacterium Planktothrix rubescens, in a large, canyon-shaped, prealpine lake (Lake Zurich, Switzerland), in six vertical profiles along a 21.7-km longitudinal transect. Highest total densities and proportions of cells with high nucleic acid content were in the warm epilimnion and the hypoxic zone of the hypolimnion. P. rubescens formed a dense layer in the metalimnion throughout the lake, whereas picocyanobacteria populated the water layers above. The epilimnion was mainly inhabited by ultramicrobacteria related to the LD12-lineage of Alphaproteobacteria and to Actinobacteria; the latter group preferred the shallow regions. Cytophaga-Flavobacteria, in particular a population related to Fluviicola sp. were more frequent in and below the layer of maximal P. rubescens abundances. Betaproteobacteria, on the other hand, were highly abundant in the epi- and hypolimnion, but not in the P. rubescens layer. Four betaproteobacterial subpopulations with contrasting longitudinal and/or vertical habitat preferences were distinguished: putatively methylotrophic bacteria of the LD28 lineage (beta IV) preferentially inhabited the hypolimnion, Polynucleobacter acidiphobus was found throughout the epilimnion, Limnohabitans (R-BT065) more in the shallow regions of the lake, and Polynucleobacter necessarius ssp. asymbioticus only in hypoxic waters. Our results stress the potential importance of spatial niche differentiation in freshwater bacterioplankton.

DOI: https://doi.org/10.4319/lo.2011.56.6.2027

Limnol. Oceanogr., 56(6), 2011, 2027–2039

doi:10.4319/lo.2011.56.6.2027

Vertical and longitudinal distribution patterns of different bacterioplankton populations in a canyon-shaped, deep prealpine lake

Michaela M. Salcher,* Jakob Pernthaler, Nakita Frater, and Thomas Posch

Limnological Station, Institute of Plant Biology, University of Zurich, Kilchberg, Switzerland

Abstract

The pelagic zone of large, deep freshwater lakes features pronounced horizontal and vertical gradients of physicochemical parameters, which in turn might allow for a nonuniform occurrence of specifically adapted bacterial taxa. We, therefore, studied the spatial distribution patterns of different heterotrophic bacteria, picocyanobacteria, and the dominant primary producer, the filamentous cyanobacterium *Planktothrix rubescens*, in a large, canyon-shaped, prealpine lake (Lake Zurich, Switzerland), in six vertical profiles along a 21.7-km longitudinal transect. Highest total densities and proportions of cells with high nucleic acid content were in the warm epilimnion and the hypoxic zone of the hypolimnion. *P. rubescens* formed a dense layer in the metalimnion throughout the lake, whereas picocyanobacteria populated the water layers above. The epilimnion was mainly inhabited by ultramicrobacteria related to the LD12-lineage of *Alphaproteobacteria* and to *Actinobacteria*; the latter group preferred the shallow regions. *Cytophaga–Flavobacteria*, in particular a population related to *Fluviicola* sp. were more frequent in and below the layer of maximal *P. rubescens* abundances. *Betaproteobacteria*, on the other hand, were highly abundant in the epi- and hypolimnion, but not in the *P. rubescens* layer. Four betaproteobacterial subpopulations with contrasting longitudinal and/or vertical habitat preferences were distinguished: putatively methylotrophic bacteria of the LD28 lineage (beta IV) preferentially inhabited the hypolimnion, *Polynucleobacter acidiphobus* was found throughout the epilimnion, *Limnohabitans* (R-BT065) more in the shallow regions of the lake, and *Polynucleobacter necessarius* ssp. *asymbioticus* only in hypoxic waters. Our results stress the potential importance of spatial niche differentiation in freshwater bacterioplankton.

There is increasing evidence that planktonic microorganisms are not homogenously distributed in pelagic realms, but rather show spatial (i.e., vertical and longitudinal) dispersal patterns. Such heterogeneities can range from microscale patchiness (μm to mm scale; Blackburn et al. 1998) to distinct global distribution patterns in the open ocean (Hoppe et al. 2002; Zwirglmaier et al. 2008).

Vertical gradients in temperature and light shape the microbial assemblages in the sea (Johnson et al. 2006; Zwirglmaier et al. 2008) and, even more pronounced, in lakes (Salcher et al. 2010; Van Den Wyngaert et al. 2011). Phototrophic microbes such as picocyanobacteria (Johnson et al. 2006; Zwirglmaier et al. 2008; Van Den Wyngaert et al. 2011), and aerobic anoxygenic phototrophs (Salka et al. 2008), or rhodopsin-bearing heterotrophic bacteria (Giovannoni et al. 2005; Sharma et al. 2009) are usually only abundant in the photic zone of oceans and lakes. Moreover, many eukaryotic algae in the well-illuminated surface-water layers are accompanied by a distinct bacterial community thriving on phytoplankton exudates (Grossart et al. 2005). On the other hand, oxygen gradients in stratified lakes provide niches for specialized microorganisms adapted to hypoxic habitats (Dimitriu et al. 2008; Salcher et al. 2008).

Although microbial niche separation along vertical gradients is well-studied, much less is known about the interplay of vertical and horizontal distribution patterns in lakes. Longitudinal heterogeneity in marine environments is mainly found along large distances and is related to transitions between waterbodies that result in environmental changes such as temperature, salinity, or nutrient gradients (Hoppe et al. 2002; Johnson et al. 2006; Schattenhofer et al. 2009). Especially estuaries offer a variety of habitat types ranging from (often nutrient-rich) lacustrine, to brackish and truly marine environments (with mostly oligotrophic conditions) that are reflected in pronounced shifts in the taxonomic composition of bacteria (Bouvier and del Giorgio 2002; Kirchman et al. 2005). Some freshwater systems, especially rivers and canyon-shaped reservoirs are also characterized by longitudinally differing nutrient regimes with changing importance of allochtonous and autochtonous carbon sources (Winter et al. 2007; Šimek et al. 2011). Reservoirs may show net heterotrophy at the riverine inflow, which eventually changes to a more phototrophic (autochtonous) assemblage in the wider and deeper areas close to the dam (Comerma et al. 2003; Šimek et al. 2008). Other, natural freshwater bodies such as large lakes are also known to differ in the longitudinal distribution of biotic and abiotic properties (Rinke et al. 2009), including the activity of the pelagic microbial assemblages (Reichart and Simon 1996).

Lake Zurich is a large (28 km), deep (136 m), oligo-mesotrophic lake, characterized by persistent annual blooms of the cyanobacterium *Planktothrix rubescens* (Bossard et al. 2001). This filamentous cyanobacterium (mean size $\sim$ 500 μm) contains several toxic secondary metabolites (e.g., hepatotoxic microcystins) as an effective protection against grazing by zooplankton, and these metabolites are also considered as harmful for humans (Blom et al. 2006). *P. rubescens* is adapted to low light irradiances and usually forms dense layers in 10–15-m

* Corresponding author: msalcher@limnol.uzh.ch

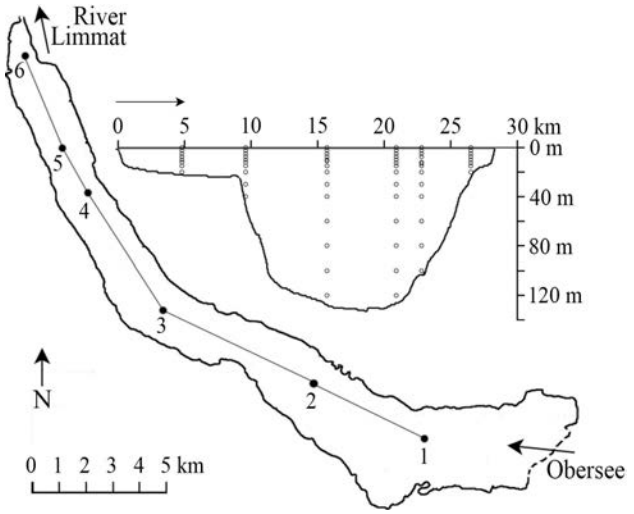


Fig. 1. Map and depth profile of Lake Zurich, Switzerland, with the position of the sampling stations (open circles) and the direction of the water flow (arrows).

depth (i.e., in the metalimnion) in late summer to autumn (Van Den Wyngaert et al. 2011). It is not yet clear whether the persistent layer of *P. rubescens* is a lake-basin–wide phenomenon or whether it forms local blooms. In this study we aimed to concomitantly identify longitudinal and vertical patterns in the respective distributions of different microbes in order to apprehend the magnitude of the 'real' (i.e., three-dimensional) variability of their metapopulations at a single time point. Furthermore, we also wanted to reveal possible correlations between the occurrence of *P. rubescens* and of different groups of heterotrophic bacteria.

Methods

Sampling—Sampling on Lake Zurich took place on 2 consecutive d (05 and 06 Aug 2009) with stable sunny weather conditions. Six stations (Sta.) along a 21.7-km transect (Fig. 1) were sampled and vertical fine-scale profiles of temperature, conductivity, oxygen content, turbidity, and chlorophyll *a* (Chl *a*) content were recorded using a YSI multiprobe (Yellow Springs Instruments, model 6600) and a fluoroprobe (TS-16-12, bbe Moldaenke GmbH), respectively. The fluoroprobe had been calibrated to distinguish different phytoplankton groups (i.e., diatoms, cryptophytes, and green algae) and *P. rubescens* according to their pigments. Water samples were taken from 0-, 2.5-, 5-, 7.5-, 10-, 15-, and 20-m, and from 30-, 40-, 60-, 80-, 100-, and 120-m depth, depending on the actual maximum depth of the respective sampling station. Additionally, one sample each was taken from the maximum depth of Chl *a* (between 11 and 12.5 m, determined via fluoroprobe), representing the dense layer of *P. rubescens*. A total number of 67 water samples were fixed onboard and stored cool until transportation to the lab. Forty mL of water were fixed with formaldehyde (2% final concentration) for flow cytometry, and 5 mL were fixed with freshly prepared buffered paraformaldehyde (pH 7.4, 2% final concentration) for analyses by fluorescence in situ hybridization followed by catalyzed reporter deposition (CARD-FISH).

Chemical analyses of water samples of the Sta. 1, 3, and the outflow river Limmat were done by the Zurich Water Supply Company. Dissolved and total phosphorus (PO_4), nitrate (NO_3), ammonia (NH_4), and dissolved and total organic carbon (DOC and TOC) were analyzed according to standard techniques.

Abundances of auto- and heterotrophic prokaryotes, and relative nucleic acid content—Total bacterial abundances and of autotrophic picocyanobacteria (presumably *Synechococcus* sp.) were counted by an inFlux V-GS cell sorter (Becton Dickinson) equipped with a ultraviolet (UV) laser (Lightwave Electronics, CY-PS, 60 mW, wavelength of 355 nm), a blue laser (Coherent, Saphire, 200 mW, wavelength of 488 nm), and detectors for two scatter and six fluorescence channels. All samples were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 μg mL^{-1} final concentration), and scatter plots were analyzed with the software FlowJo 7.2.2. (Tree Star). Bacteria with high and low nucleic acid content (HNA and LNA bacteria, respectively) were discriminated by their respective desoxyribonucleic acid (DNA) fluorescence. HNA and LNA populations were clearly distinguishable in all samples.

Abundances of different phylogenetic groups of bacteria—CARD-FISH was carried out as previously described (Amann and Fuchs 2008) with slight modifications for probe LD12-121 (Salcher et al. 2011). The following HRP labeled oligonucleotide probes were used: EUB I–III, detecting all *Bacteria*, HGC69a for *Actinobacteria*, CF319a detecting most *Cytophaga–Flavobacteria* of *Bacteroidetes* (CF), BET42a for *Betaproteobacteria* (Amann and Fuchs 2008), LD12-121 for the LD12 lineage of *Alphaproteobacteria* (Salcher et al. 2011). Probe Flu-736 was newly designed to target a small cluster of microbes affiliated to *Fluviicola* sp. (Table 1). *Betaproteobacterial* subpopulations were identified by the following probes: R-BT065, detecting a cluster related to the genus *Limnohabitans* (Šimek et al. 2001), PnecB-23S-116, and PnecC-445 (Wu and Hahn 2006*a*) for the discrimination of two species of the genus *Polynucleobacter*. In addition, probe LD28-1017 was newly designed to target a monophyletic branch of uncultivated bacteria from the LD28 (beta IV) lineage (Table 1). Signal amplification was done with fluorescein- or Alexa488-labeled tyramides. CARD-FISH stained filters were analyzed by fully automated high-throughput microscopy (Zeder and Pernthaler 2009), recording three pictures of the same image section: UV excitation for DAPI-stained cells, blue excitation for hybridized cells, and green excitation for autofluorescent objects (picocyanobacteria and debris) that would otherwise interfere with hybridization signals. All images were analyzed with an image analysis macro (M. Zeder unpubl.), and interfering autofluorescent objects (cyanobacteria or debris) were individually subtracted from pictures of hybridized cells. At least

Table 1. Oligonucleotide probes designed in this study. FA% = Formamide concentrations.

Probe name	Specificity	Target hits (coverage), false positive hits	Sequence (5′ to 3′)	FA%
LD28-1017	Freshwater LD28-clade (*Methylophilaceae*)	112 (98.1%), 1 *Acidivorax*	TCT CTT TCG AGC ACT TGA ACA	45
LD28-C	Competitor for LD28		TCT CTT TCG AGC ACT TTC ACA	—
LD28-H1-999	Helper 1 for LD28		TCT CTG CTC AAT TCG GTA	—
LD28-H2-1036	Helper 2 for LD28		CAG CAC CTG TGT TAC CGT	—
LD28-H3-1054	Helper 3 for LD28		GAG CTG ACG ACA GCC ATG	—
LD28-H4-1072	Helper 4 for LD28		CCC AAC ATC TCA CGA CAC	—
Flu-736	*Fluviicola* sp. (*Cryomorphaceae*)	148 (80.5%)	CAA TYC AGG CCT AGT GAG	45

10 high-quality images or at least 1000 DAPI-stained particles (mean = 3866, minimum = 1048, maximum = 11,220) were analyzed for each sample. Replicated hybridization of 89 samples (randomly chosen filters hybridized with different probes) resulted in a mean standard deviation of ± 1.0% of DAPI counts (coefficient of variation [CV] = 0.16, n = 39) for larger populations (i.e., > 5% of DAPI counts) and ± 0.24% of DAPI (CV = 0.18, n = 50) for small populations.

Phylogenetic analyses and probe design of 16S ribosomal ribonucleic acid (rRNA) genes was done with sequences obtained during autumn 2007 (water samples from 10-, 12.5-, and 15-m depth; Van Den Wyngaert et al. 2011; Salcher et al. 2011). Four hundred forty-six sequences of Lake Zurich were deposited to the European Molecular Biology Laboratory with the accession no. FN665702–FN665785 and FN668013–FN668374 (*see* Van Den Wyngaert et al. [2011] and Salcher et al. [2011] for more detail). Probe design was carried out with the ARB software package (Ludwig et al. 2004). Only high-quality sequences > 1200 nucleotides were used for tree reconstruction. Maximum parsimony, neighbor-joining and maximum-likelihood analyses were performed with the respective ARB tools. The resulting trees were compared manually to obtain a consensus tree. Probe design for the LD28 and the *Fluviicola* sp. cluster was done with the respective ARB tools Probe_Design and Probe_Check, and probe candidates were also checked for specificity in the ribosomal database project (http://rdp.cme.msu.edu). Four helper oligonucleotides and a competitor were designed for probe LD28-1017 to obtain higher accessibility to the 16S rRNA region and to discriminate nontarget organisms (Fuchs et al. 2000). The resulting probes were tested with different formamide concentrations until highest specificity was achieved (Table 2).

Statistical analysis—All statistical analyses were done with log(+1)- and arcsine-transformed raw data (environmental and percentage data, respectively) to ensure normal distribution. One-way ANOVAs were done to test for significant differences between epi- (0–7.5-m depth, n = 24), meta- (10–15-m depth, n = 18), and hypolimnion (20–120-m depth, n = 25) samples. Canonical correspondence analysis was used to determine the effects of depth and environmental variables on the spatial distribution of the bacterial populations at different stations. Only those environmental variables exhibiting a significant correlation ($p < 0.001$) to bacterial populations were included in the analysis. The significance of added variables was tested by a Monte-Carlo permutation test (500 permutations, $p < 0.001$). Analyses were computed with the Microsoft EXCEL add-in program XLSTAT-ADA (www.xlstat.com).

Results

Lake Zurich showed a stable summer stratification in August 2009, with warm epilimnetic water (22.2° ± 0.3°C in 1-m depth along the whole transect), and the metalimnion (> 1°C difference m^{-1}) was located between 9-m and 17-m depth. The metalimnion was characterized by a steep decrease in light intensities and a peak of Chl *a* concentrations (Fig. 2A,B). Most of this Chl *a* was related to the cyanobacterium *Planktothrix rubescens* (up to 27.6 μg Chl *a* L^{-1}), which formed a dense layer in 11–13-m depth (Fig. 2C). Diatom-related Chl *a* (Fig. 2D; up to 3.1 μg Chl *a* L^{-1}, mainly *Asterionella* sp. and *Fragilaria* sp.), as well as coccoid picocyanobacteria (Fig. 2E; presumably *Synechococcus* sp.), were only present in depth layers above this *P. rubescens* bloom. These two groups of primary producers moreover showed a clear longitudinal trend, with signifi-

Table 2. Chemical parameters at Sta. 1 and 3 (mean of 0–20-m depths) and at the outflow of Lake Zurich (surface) at 03–05 August 2009.

Parameter	Sta. 1	Sta. 3	Outflow (Limmat)
Dissolved phosphorus (μg L^{-1})	<2	<2	<2
Total phosphorus (μg L^{-1})	14.7	13.8	16.0
Nitrate (mg L^{-1})	1.91	1.86	0.93
Ammonia (μg L^{-1})	17.8	12.8	12.0
Dissolved organic carbon (mg L^{-1})	1.19	1.46	1.48
Total organic carbon (mg L^{-1})	2.05	2.12	1.8

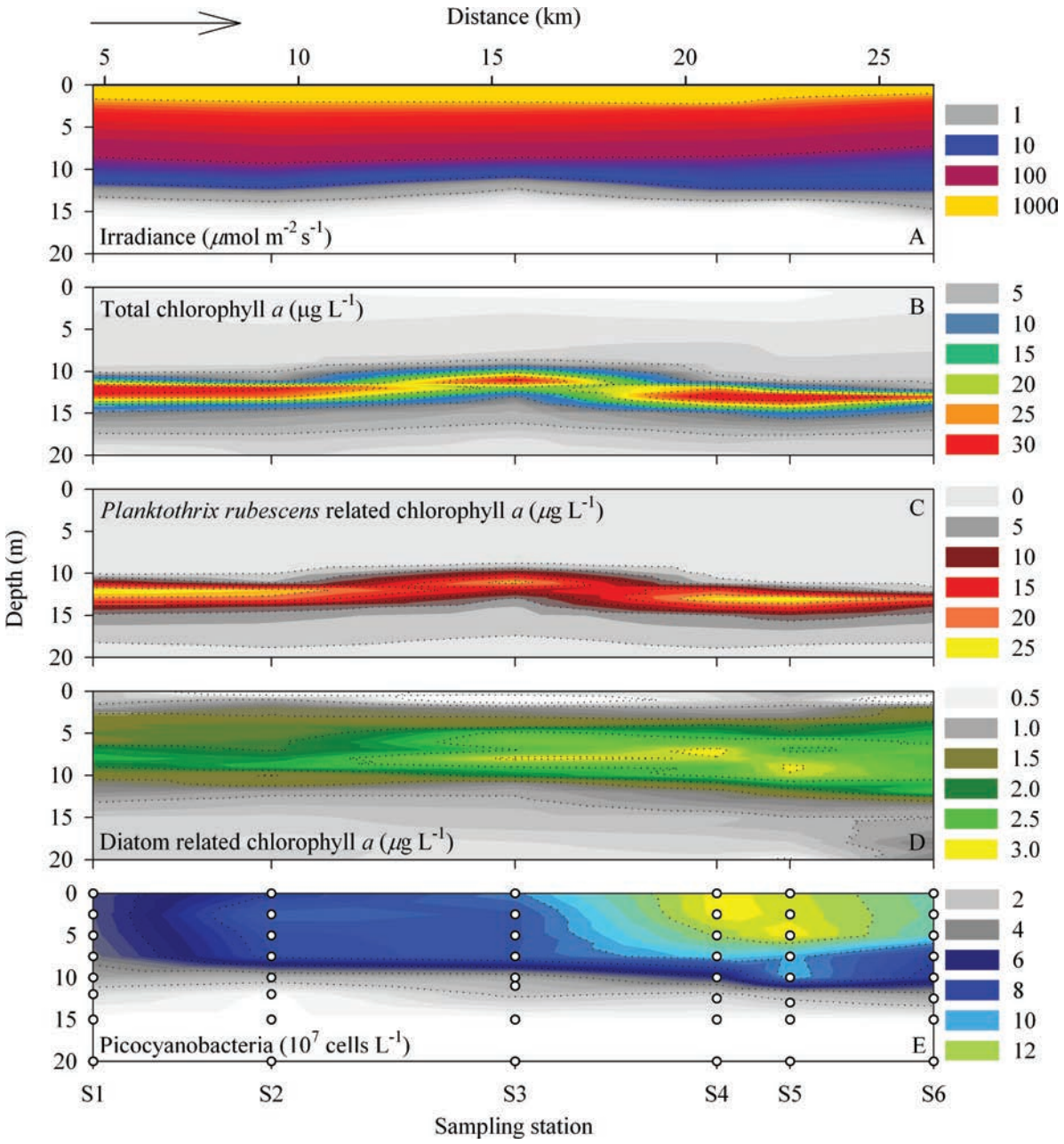


Fig. 2. (A) Irradiance (μmol m^{-2} s^{-1}), (B) total concentrations of Chl *a* (μg L^{-1}), (C) *Planktothrix rubescens*–related Chl *a* (μg L^{-1}), (D) diatom-related Chl *a* (μg L^{-1}), and (E) picocyanobacterial abundances (10^7 cells L^{-1}) in 0–20-m depth along the sampling transect. Circles indicate sampling depths and the arrow points to the direction of the water flow.

cantly higher densities in the middle and toward the outflow of the lake (Fig. 2D,E). Heterotrophic bacteria, on the other hand, were significantly more abundant in the inflow region, with up to 3.8×10^9 cells L^{-1} at Sta. 2 (Fig. 3B). Bacterial abundances dropped to mean densities of $1.3 \pm 0.3 \times 10^9$ cells L^{-1} in the hypolimnion, but increased again above the sediment in 100-m and 120-m depths at Sta. 3–5. These sampling points were characterized by hypoxic conditions (i.e., oxygen saturation of $< 30\%$ [Fig. 3A; 1.8 and 1.0 mg O_2 L^{-1} in 120-m depth at Sta. 3 and 4, and 2.96 mg O_2 L^{-1} in 99-m depth at Sta. 5]). At Sta. 5 we took the 100-m sample just above the sediment, while at all other stations at least 2 m of distance between the last sampling depth and the sediment were recorded. More than one-quarter ($28.2\% \pm 4.2\%$) of all bacteria were of HNA content, with slightly higher values for the hypolimnion and a pronounced peak at the hypoxic Sta. 5 in 100-m depth (51%; Fig. 3C). Around half of all DAPI-stained objects were hybridizable with the general bacterial probe EUB I–III ($50.2\% \pm 6.5\%$, data not shown), with a maximum in 100 m at Sta. 5 (73.9% of DAPI). By applying three general probes for *Actinobacteria* (HGC69a), *Betaproteobacteria* (BET42a), and CF (CF319a), and the specific probe for the LD12 cluster of

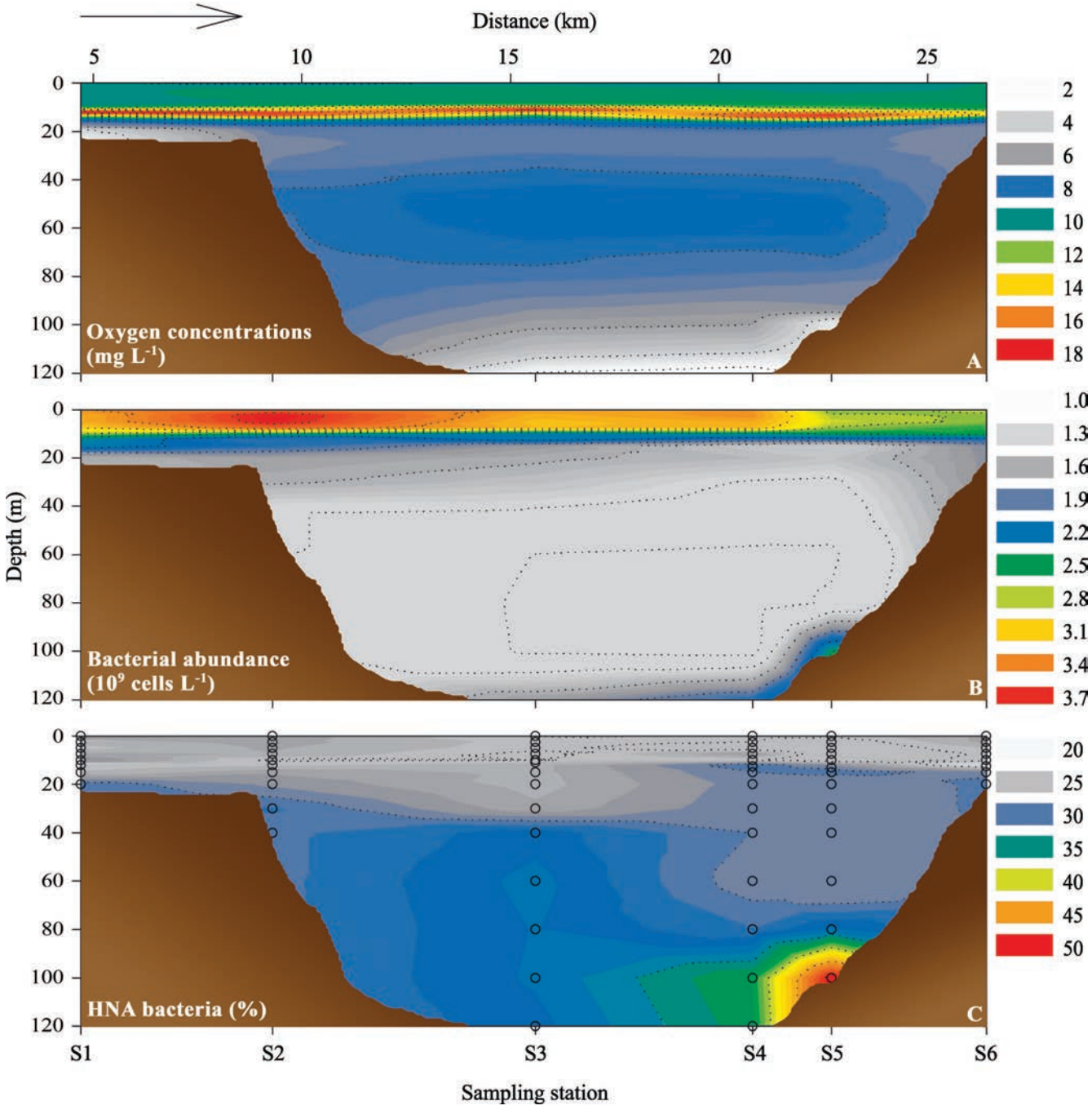
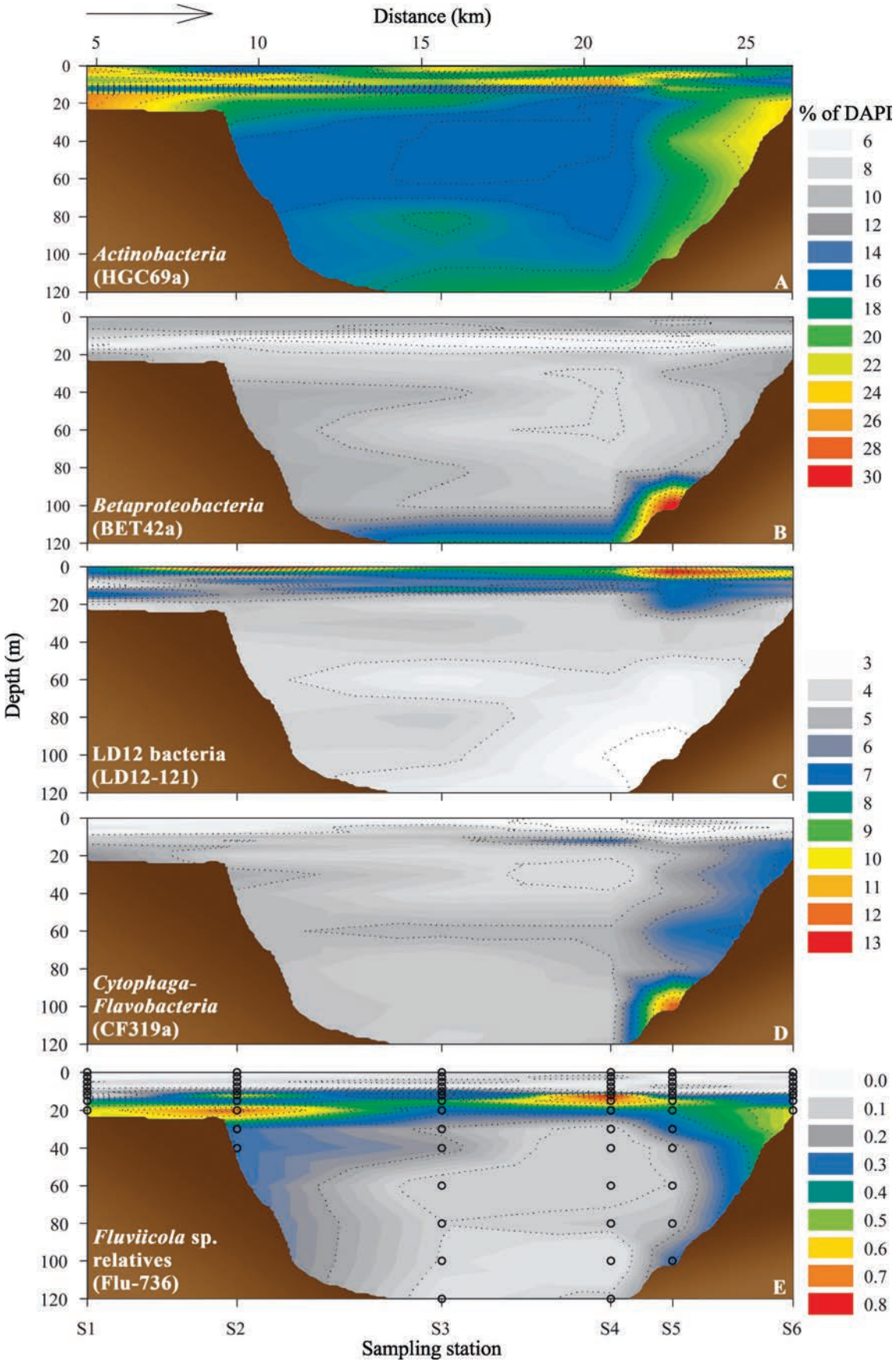


Fig. 3. (A) Oxygen concentrations (mg L^{-1}), (B) bacterial abundances (10^9 cells L^{-1}), and (C) relative HNA abundances (% of DAPI) in 0–120-m depth along the sampling transect. Open circles indicate sampling depths and the arrow points to the direction of the water flow.

Alphaproteobacteria (LD12-121), we identified 89% ± 7.2% of all *Bacteria* hybridized with probe EUB I–III.

Relative abundance of different bacterial populations—The most abundant microbes were *Actinobacteria* (mean 18.8% ± 4.2% of DAPI) with higher relative densities in the warm epilimnion, but also with a very patchy horizontal distribution (Fig. 4A). *Betaproteobacteria* were highly abundant in the hypoxic samples in the deep hypolimnion (up to 32% of DAPI; Fig. 4B), and showed a clear vertical pattern with significantly lower proportions in the metalimnion compared to the epi- and hypolimnion (10.6%, 6.4%, and 10.9% of DAPI in the epi-, meta-, and hypolimnion, respectively; one-way ANOVA: $F_{66} = 11.5$, $p < 0.001$). No clear longitudinal trend was found, except for the peaks in the hypoxic layers. Uncultured bacteria of the freshwater LD12-lineage were highly abundant only in the warm epilimnion (mean relative densities = 8.3% ± 2.5%


Distance (km)
5
10
15
20
25
Depth (m)
0
20
40
60
80
100
120
Actinobacteria
(HGC69a)
A
Betaproteobacteria
(BET42a)
B
LD12 bacteria
(LD12-121)
C
Cytophaga-
Flavobacteria
(CF319a)
D
Fluviicola sp.
relatives
(Flu-736)
E
% of DAPI
6
8
10
12
14
16
18
20
22
24
26
28
30
3
4
5
6
7
8
9
10
11
12
13
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
S1
S2
S3
S4
S5
S6
Sampling station

of DAPI), while less than half of these proportions were present in the hypolimnion (one-way ANOVA: $F_{66} = 36.4$, $p < 0.001$). These microbes showed a patchy longitudinal distribution with peaks of > 13% of DAPI at Sta. 2 and 5 (Fig. 4C). Bacteria affiliated with CF also showed a vertical density gradient, with significantly less percentages in the epi- compared to the meta- and hypolimnion (Fig. 4D; one-way ANOVA: $F_{66} = 13.8$, $p < 0.001$). As was also observed for *Betaproteobacteria*, CF had a pronounced peak in the hypoxic samples (12.4% of DAPI). The newly designed probe Flu-736, targeting 148 almost complete 16S rRNA gene sequences of the family *Cryomorphaceae* (132 *Fluviicola* sp., 3 *Lishzhenia* sp., 2 *Crocinitomix* sp., 9 *Brumimicrobium* sp., and 2 unclassified *Cryomorphaceae*; Fig. 5; Table 1), accounted for almost 10% of all CF in 12–20-m depths. These microbes were virtually absent in the epilimnion (0.06% of DAPI) and below 30 m (0.14% of DAPI), but were 6–9-fold more abundant in the depth layer of *P. rubescens* and in 15-m and 20-m depth (Fig. 4E).

Relative abundance of betaproteobacterial populations—We identified 56.6% of all *Betaproteobacteria* (range = 25.2–91.2%) with four genus- to species-specific probes (Fig. 6). Members of the *Limnohabitans* sp. cluster (probe R-BT065) were most abundant (24% of *Betaproteobacteria*), followed by the LD28 cluster (probe LD28-1017, 18.1% of *Betaproteobacteria*), and the species *Polynucleobacter acidiphobus* (probe PnecB-23S-166, 11.7% of *Betaproteobacteria*), and *Polynucleobacter necessarius* ssp. *asymbioticus* (probe PnecC-445, 3% of *Betaproteobacteria*). *Limnohabitans* sp. were distributed all over the lake but with pronounced peaks in the epilimnion at the in- and outflow regions (Fig. 6A). The newly designed probe LD28-1017 targets 112 almost full-length 16S rRNA gene sequences of the family *Methylophilaceae*, whereof 102 were affiliated with the monophyletic freshwater cluster LD28 (98% group coverage). Three more sequences of the closely related marine OM43 cluster and seven unclassified *Methylophilacea* were also targeted by the probe, as well as one false-positive sequence affiliated with *Acidivorax*. Bacteria hybridizing with probe LD28-1017 occurred at significantly higher relative abundances in the hypolimnion (Fig. 6B; 2.0% ± 0.4% compared to 1.1% ± 0.3% of DAPI in the epilimnion; one-way ANOVA: $F_{66} = 42.7$, $p < 0.001$), while *P. acidiphobus* showed the opposite distribution (Fig. 6C; 2.0% ± 0.6% in the epilimnion compared to 0.7% ± 0.2% of DAPI in the hypolimnion; one-way ANOVA: $F_{66} = 70.0$, $p < 0.001$). *P. necessarius* ssp. *asymbioticus* was virtually absent in most samples (0.2% ± 0.1% of DAPI), except for the three deep hypolimnetic samples with hypoxic conditions (Fig. 6D; up to 4.5% of DAPI).

The chemical analyses of water samples from Sta. 1, 3, and at the outflow of the river Limmat revealed a gradient of nitrate (NO_3), ammonia (NH_4), and DOC (Table 2). Highest concentrations of nitrate (1.91 mg L^{-1}) and ammonia (17.8 μg L^{-1}) and lowest concentrations of DOC (1.19 mg L^{-1}) were measured at the inflow region of the lake.

Influence of environmental parameters on the distribution of microbes—Canonical correspondence analysis revealed a significant relationship between environmental parameters and bacterial populations (pseudo-$F_{66} = 1.95$, $p < 0.0001$), where axis 1 explained 52.98% and axis 2 another 32.43% of the total variation (Fig. 7A). A clear clustering of epi-, meta-, and hypolimnetic samples, and therein a separation in oxic and hypoxic hypolimnetic samples was detected (Fig. 7A). Axis 1 separated epi- and metalimnetic samples from hypolimnetic samples, and axis 2 divided metalimnetic and oxygenated hypolimnetic samples from epilimnetic and hypoxic hypolimnetic samples. The variation along axis 1 was mostly associated with sampling depth, while the chlorophyll concentration of *P. rubescens* was most significant on axis 2. Microbes affiliated with *P. acidiphobus* (PnecB) and LD12 clustered together with epilimnetic samples and were positively related to light intensities and temperature and, to a lesser extent, to diatom chlorophyll. The close relationship of metalimnetic samples with *Limnohabitans* sp. (R-BT) was mainly related to oxygen content, while *Fluviicola* sp. (Flu) were influenced exclusively by the chlorophyll of *P. rubescens*. Microbes affiliated with LD28 and CF were situated in the cluster of oxygenated hypolimnetic samples and correlated with conductivity and depth layer, while *P. necessarius* ssp. *asymbioticus* (PnecC) clustered together with samples of the hypoxic hypolimnion and were positively related to depth and negatively to oxygen content (Fig. 7A). All bacterial populations except *Limnohabitans* sp. (R-BT) showed a higher vertical than longitudinal CV if all samples were pooled (data not shown). However, the opposite was found when only the epilimnetic samples were considered (Fig. 7B). Most pronounced differences in the longitudinal distribution were identified for total heterotrophic bacteria, picocyanobacteria, *Limnohabitans* sp. (R-BT), and *Actinobacteria* (HGC).

Discussion

Vertical niche separation of prokaryotes—Steep vertical physicochemical gradients divided Lake Zurich in four separated compartments (epilimnion, metalimnion, oxic, and hypoxic hypolimnion) in August 2009 (Fig. 7A). The warm epilimnion was inhabited by diatoms, picocyanobacteria (Fig. 2D,E), and ultramicrobacteria affiliated with *Actinobacteria*, LD12, and *Polynucleobacter acidiphobus* (Figs. 4A,C; 6C). Recently, the freshwater SAR11 lineage LD12 was shown to be highly abundant in the epilimnion

←

Fig. 4. Relative abundances (% of DAPI) of (A) *Actinobacteria*, (B) *Betaproteobacteria*, (C) LD12 bacteria, (D) *Cytophaga–Flavobacteria*, and (E) microbes affiliated with *Fluviicola* sp. in 0–120-m depth along the sampling transect. Open circles indicate sampling depths and the arrow points to the direction of the water flow.

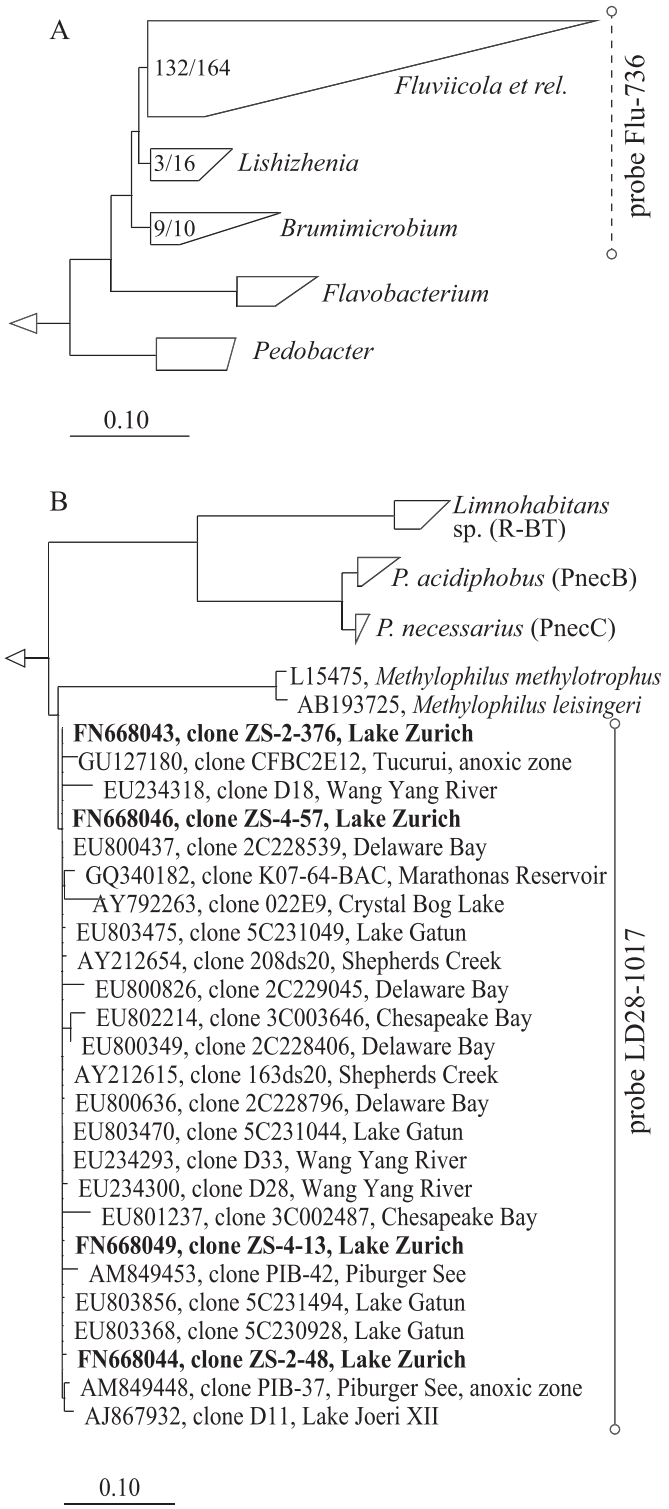


Fig. 5. Unrooted phylogenetic consensus trees of 16S rRNA genes of (A) *Cryomorphaceae* and relatives (including probe Fluv-736), and (B) *Betaproteobacteria* (including probe LD28-1017). Clone sequences in bold are derived from Lake Zurich. The scale bars represent 10% estimated sequence divergence. (A) Numbers indicate the number of sequences targeted by probe Fluv-736 and total sequence size of each clade.

of Lake Zurich in summer, possibly favored by high water temperatures, light, and low nutrient concentrations (Salcher et al. 2011). Similarly, *P. acidiphobus* were found to thrive in the warm realms of two large lakes (Wu and Hahn 2006*a*, *b*). *Actinobacteria* were present in the whole water column of Lake Zurich, but with pronounced preferences for the epi- and metalimnion, as was also observed in other European lakes (Allgaier and Grossart 2006; Salcher et al. 2010). Several *Actinobacteria* contain light-harvesting pigments termed actinorhodopsins (Sharma et al. 2009). Atamna-Ismaeel et al. (2008) have identified freshwater proteorhodopsins possibly belonging to LD12 bacteria, providing a plausible possible explanation for their high densities in the light-penetrated epilimnion. The epilimnion of Lake Zurich is usually nutrient-depleted during summer (Table 2; Salcher et al. 2011) and the minute cell sizes of *Actinobacteria* (Salcher et al. 2010), LD12 (Salcher et al. 2011), and *P. acidiphobus* (Hahn et al. 2011) might provide an effective adaptation to these situations, because their optimized surface : volume ratio points to advantages in nutrient and substrate uptake (Giovannoni et al. 2005). Moreover, bacterivorous nanoflagellates and ciliates are typically more abundant in the epilimnion of Lake Zurich (M. Salcher unpubl.), and the small cell sizes of these bacteria might provide an efficient grazing protection (Pernthaler 2005). Although we did not estimate the effect of grazing in this study, it would be of interest to include such data in further surveys to gain more insight in the interplay of the patchy spatial occurrence patterns of epilimnetic bacteria (Fig. 7B) and their main grazers.

The metalimnion was dominated throughout the lake by the harmful cyanobacterium *Planktothrix rubescens* (Fig. 2C). Although other typical primary producers such as diatoms or cryptophytes are known to favor distinct bacterial populations within *Betaproteobacteria* (Šimek et al. 2008), CF (Zeder et al. 2009), or *Actinobacteria* (Salcher et al. 2010), this seems to be only partially the case for *P. rubescens*. Significantly lower proportions of *Betaproteobacteria* were present in the layers of the cyanobacterium compared to the epi- and hypolimnion (Fig. 4B). A negative effect of cyanobacterial blooms on *Betaproteobacteria*, especially on *Limnohabitans* sp., was also detected in a eutrophic reservoir (Hornák et al. 2008). It is not yet clear whether secondary metabolites released by *P. rubescens* negatively affect microbes or whether the very low DOC excretion rates of these cyanobacteria are not sufficient for a stimulation of the bacterial assemblage (Feuillade et al. 1988). As suggested by Van Den Wyngaert et al. (2011), *P. rubescens* might even act as a competitor of auto- and heterotrophic microbes for nutrient and carbon sources. Only CF, especially bacteria related to *Fluviicola* sp. were consistently more abundant in the cyanobacterial layer (Fig. 4D,E). A beneficial effect of cyanobacterial blooms on CF has been described previously (Eiler and Bertilsson 2004; Hornák et al. 2008) and 16S rRNA gene sequences affiliated with CF were highly abundant in samples from the *P. rubescens* layer in Lake Zurich (Van Den Wyngaert et al. 2011). In that study, several sequence types affiliated with *Fluviicola* sp. were unique for this layer

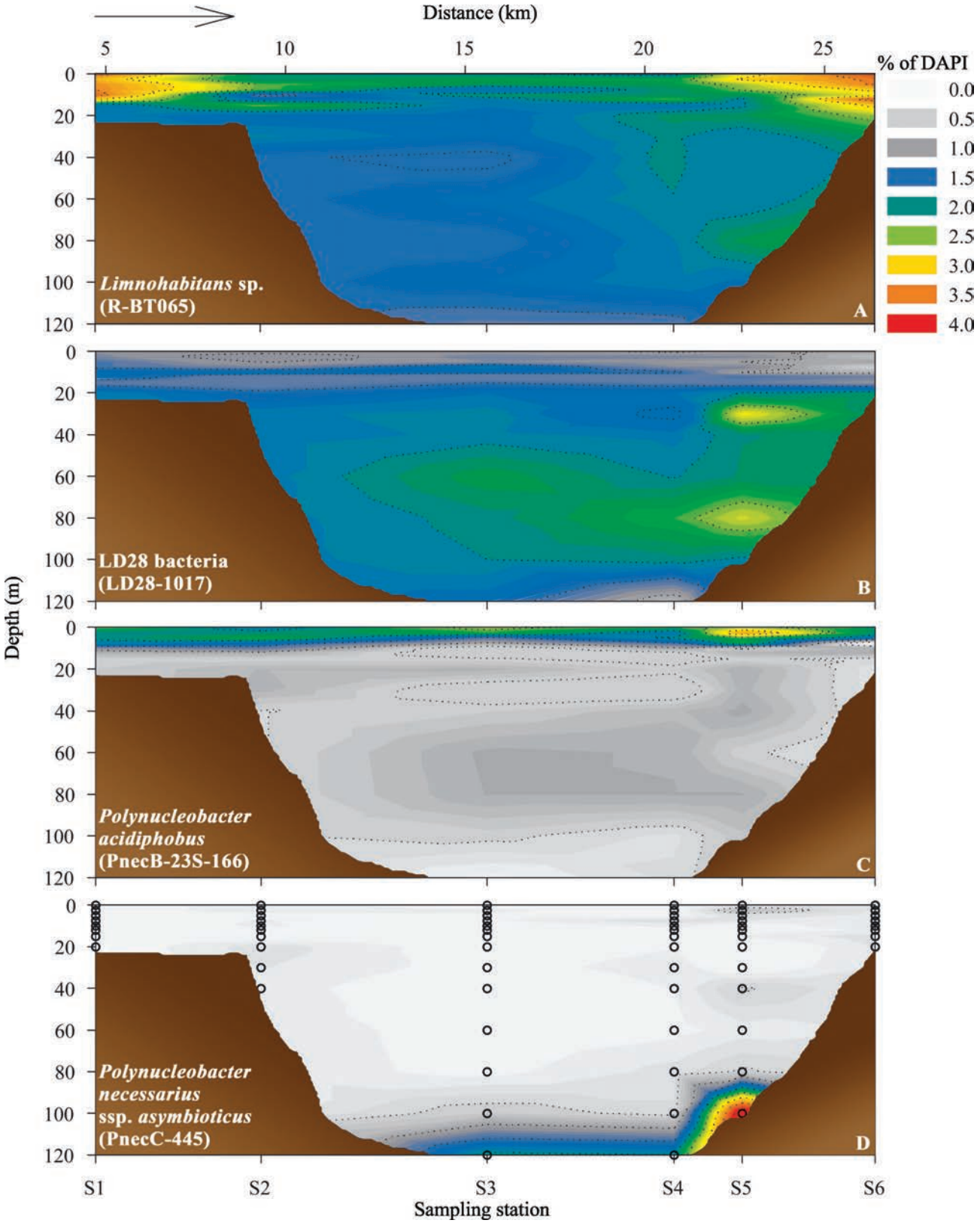


Fig. 6. Relative abundances (% of DAPI) of microbes affiliated with (A) *Limnohabitans* sp., (B) uncultured LD28, (C) *Polynucleobacter acidiphobus*, and (D) *P. necessarius* ssp. *asymbioticus* in 0–120-m depth along the sampling transect. Open circles indicate sampling depths and the arrow points to the direction of the water flow.

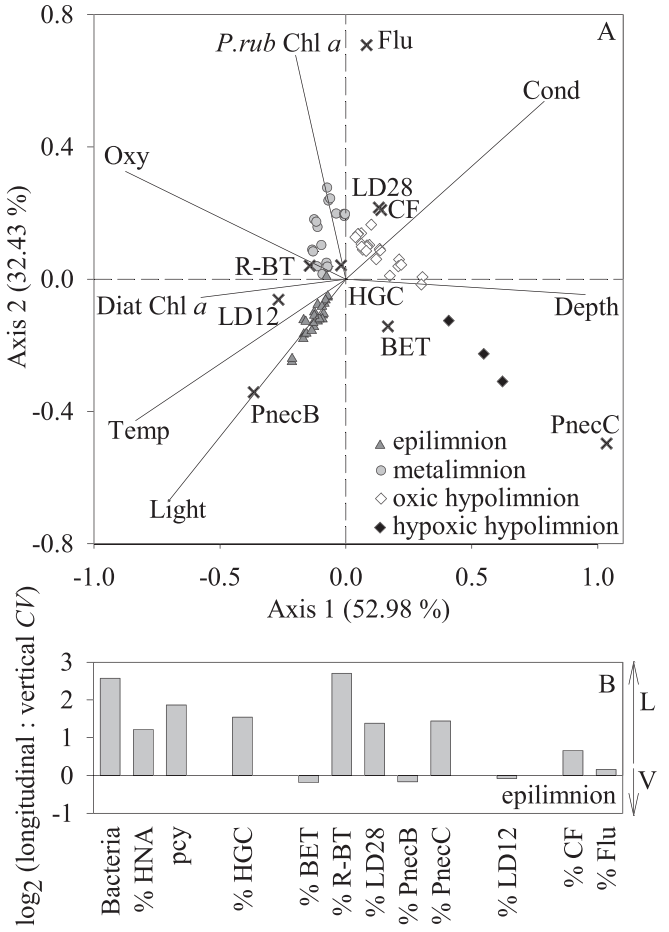


Fig. 7. (A) Canonical correspondence analysis of heterotrophic bacteria in Lake Zurich. (B) Longitudinal to vertical coefficient of variation (CV) of all prokaryotic populations calculated for epilimnetic samples (0–7.5-m depth, $n = 24$). The arrows point to either a strong longitudinal (L) or vertical (V) difference in the distribution of the populations. *P.rub* Chl *a*: Chl *a* associated with *P. rubescens*; Diat Chl *a*: Chl *a* associated with diatoms; Temp: temperature; Cond: conductivity; Oxy: oxygen content; pcy: picocyanobacteria; Flu: *Fluviicola* sp. relatives; LD28: microbes affiliated with LD28; CF: *Cytophaga–Flavobacteria* of the *Bacteroidetes*; PnecB: *P. acidiphobus*; PnecC: *P. necessarius* ssp. *asymbioticus*; BET: *Betaproteobacteria*; HGC: *Actinobacteria*; LD12: microbes affiliated with LD12; R-BT: *Limnohabitans* sp.

(i.e., they were absent in samples from 2.5 m above or below; Van Den Wyngaert et al. 2011). These findings inspired us to construct a specific FISH probe to provide quantitative evidence for the preference of these microbes for the *P. rubescens* layer (Figs. 4E, 7A), possibly related to the degradation of specific substrates released by the cyanobacterium. Some CF are known to be highly specialized for the degradation of high molecular weight substrates (Kirchman 2002). The closest validly described species, *Fluviicola taffensis*, shows a lack of nutritional versatility, with an inability to utilize carbohydrates such as glucose (O'Sullivan et al. 2005) but grows on complex media such as Tryptone Soya agar or Plate Count agar. Interestingly, microbes targeted by probe Flu-736 were also highly abundant during a phytoplankton spring bloom in Lake Zurich, where they constituted up to 54% of all CF (E. Eckert unpubl.).

The oxygenated hypolimnion represented a more uniform compartment where almost all bacterial groups (*Actinobacteria, Betaproteobacteria*, and CF) were present. At a higher phylogenetic resolution, *Betaproteobacteria* affiliated with the LD28 cluster (Fig. 6B) preferably inhabited the deep realms of Lake Zurich. Microbes of this species-like clade within *Methylophilaceae* (also termed beta IV [Glöckner et al. 2000] or beta IVa [Salcher et al. 2008]) are typical freshwater bacteria with a ubiquitous distribution (Glöckner et al. 2000). High numbers of *Methylophilaceae* were found in the anoxic hypolimnion of an alpine lake, as identified by a more general CARD-FISH probe also targeting the typical methylotrophic genera *Methylophilus*, *Methylovorus*, and *Methylobacillus* (Salcher et al. 2008). The application of the newly designed more specific probe LD28-1017 on samples from that study revealed that these *Methylophilaceae* were in fact all affiliated with the LD28 cluster (M. Salcher unpubl.). Bacteria of the LD28 cluster were also found in fingerprinting profiles from a shallow humic lake throughout 3 consecutive yr (Newton et al. 2006). There are as yet no physiological data from cultured members of this clade; therefore, it is not yet clear whether they indeed have a methylotrophic metabolism. Only very few cells of this clade were found to be capable of amino acid incorporation in Piburger See (Salcher et al. 2008). Preliminary data from Lake Zurich suggest that these bacteria also show almost no uptake of various monomeric substrates such as fructose, acetate, thymidine, or glucose (M. Salcher unpubl.). On the other hand, LD28 bacteria have been enriched in synthetic lake water amended with a mix of non–C_1 substrates such as glucose or amino acids (Gich et al. 2005). However, axenic isolates of the closely related marine OM43 clade are obligate methylotrophic microbes oxidizing C_1 compounds like methanol and formaldehyde (Giovannoni et al. 2008). This might hint at a comparable lifestyle of LD28 bacteria.

The hypoxic samples in 100–120-m depth clearly differed from all other samples (Fig. 7A) with a pronounced peak of *Betaproteobacteria* (especially *Polynucleobacter necessarius* ssp. *asymbioticus*) and CF (Figs. 4B,D; 6D). *P. necessarius* ssp. *asymbioticus* (PnecC) harbors distinct ecotypes that favor the anoxic realms of stratified lakes (Salcher et al. 2008; Jezberová et al. 2010). These microbes were almost undetectable in the oxygenated samples of Lake Zurich (Fig. 6D). Some prokaryotes affiliated with CF are also known to thrive in hypoxic hypolimnia (Dimitriu et al. 2008; Salcher et al. 2010).

Longitudinal trends in Lake Zurich—We could show for the first time that the bloom of *P. rubescens* is a basin-wide phenomenon in Lake Zurich (Fig. 2C). Therefore, heterotrophic microbes in this layer might also be influenced at a lake-wide scale. Although the meta- and hypolimnion showed only very little longitudinal differences, this was not the case for the epilimnion (Fig. 7B). The theoretical water retention time of Lake Zurich is 1.2 yr. However,

because the main inflow is a connected lake (Obersee) with warm water temperature in summer, the incoming water is directly mixed in the epilimnion (i.e., overflow [Šimek et al. 2011]). Therefore, the actual water-renewal time during summer likely is strongly reduced in the epilimnion and prolonged in the colder water layers. This makes the epilimnion a separated ecosystem with a very patchy distribution of different microbial populations. An almost six-fold higher longitudinal than vertical CV of total bacterial numbers was detected in the epilimnion, and a more than three-fold higher one was detected for picocyanobacteria (Fig. 7B). The inflow region was dominated by heterotrophic bacteria rather than autotrophs (Fig. 3B). These findings fit with the results of the water chemical analyses (i.e., higher concentrations of nitrate and ammonia, but lower concentrations of DOC at Sta. 1 compared to the middle of the lake and the outflow [Table 2]). A tendency to net heterotrophy in inlet regions is also known from large canyon-shaped reservoirs (Šimek et al. 2008, 2011). In the central region of the lake (Sta. 3 and 4), the planktonic community changed to more autotrophy with a bloom of diatoms and a peak of picocyanobacteria (Fig. 2D,E). Concomitantly, a decrease in nitrate and ammonia and an accumulation of DOC was detected (Table 2). This trend continued toward the outflow of the lake. Here, the densities of heterotrophic bacteria decreased (Fig. 3B), but no specific bacterial population was identified to decrease in proportion in the same manner.

No single population of the studied bacterial taxa was solely responsible for the observed peak of total bacteria in the inflow. However, the most abundant microbes in the shallow inlet area were small *Actinobacteria* (Fig. 4A). These bacteria showed an extent of longitudinal patchiness (Fig. 7B) that was significantly higher than the methodological variability of the CARD-FISH counting protocol (one-way ANOVA of 15 replicated hybridizations vs. the closest longitudinal neighbors: $F_{15} = 10.72$, $p = 0.003$), and that was almost three-fold higher than the variability of their vertical distribution (Fig. 7B). Almost all *Actinobacteria* in Lake Zurich are affiliated with the ubiquitous ac1 cluster (data not shown), which harbors at least 11 subclusters with differing adaptations to environmental factors (e.g., pH; Newton et al. 2007). The observed patchiness in the relative abundance of *Actinobacteria* in the epilimnion might, thus, be related to the respective distribution patterns of such ecotypes. However, it is still unknown how many of these subclades indeed form substantial populations in Lake Zurich.

A pronounced longitudinal patchiness was also found for *Limnohabitans* sp. (> seven-fold higher longitudinal than vertical CV; Fig. 7B). In contrast to *Actinobacteria* these microbes showed a bimodal distribution, with peaks both in the in- and outflow regions (Fig. 6A). *Limnohabitans* sp. are ubiquitously distributed in a wide range of freshwater habitat types (Kasalicky et al. 2010). These microbes typically feature a so-called opportunistic growth strategy, with high growth rates closely coupled to nutrient pulses by algal exudates originated from (for example) cryptophytes (Šimek et al. 2008). On the other hand, some *Limnohabitans* sp. are highly vulnerable to grazing by heterotrophic nanoflagellates (Šimek et al. 2010). The *Limnohabitans* cluster so far comprises four described species that differ in morphology and phenotypic traits such as substrate acquisition patterns (Kasalicky et al. 2010). The well-established probe R-BT065 targets only two of these isolates (i.e., *L. parvus* and *L. planktonicus*; Kasalicky et al. 2010) with different susceptibility to protistan predation and viral lysis (Šimek et al. 2010). Therefore, ecological niche differentiation of *Limnohabitans* ecotypes might be a possible explanation for their conspicuous longitudinal distribution in Lake Zurich. Moreover, overflow events as observed in Lake Zurich have been found to favor heterotrophic situations in a reservoir, with sequential peaks of bacteria, flagellates, and ciliates along the longitudinal axis (Šimek et al. 2011). Such spatial separation of *Limnohabitans* sp. and their predators might, therefore, also be the case in Lake Zurich. For future studies it might, thus, be promising to estimate the influence of protistan grazers on the distribution patterns of different bacterioplankton populations.

Our study represents a single time snapshot of the spatial distribution of microbes in Lake Zurich. The structure of microbial assemblages is known to change seasonally (Šimek et al. 2008; Salcher et al. 2010), and individual populations may be specifically adapted to temporal changes in their abiotic and biotic environment. It is likely that microbes affiliated with (for example) LD12 and *P. acidiphobus* in the epilimnion (Figs. 4C, 6C) might not be detected in high numbers during other seasons, because these bacteria seem to be adapted to the nutrient-depleted, but warm epilimnion (Wu and Hahn 2006*b*; Salcher et al. 2011). In contrast, *Actinobacteria* might be even more important during spring and/or autumn (Glöckner et al. 2000; Allgaier and Grossart 2006; Salcher et al. 2010). Their small size and their gram-positive cell wall act as effective protection against grazing by bacterivorous protists (Pernthaler 2005) and high densities of *Actinobacteria* often co-occur with peaks of flagellates (Salcher et al. 2010). By contrast, the occurrence of *Betaproteobacteria* (and therein especially of *Limnohabitans* sp.) and CF is often positively related to algal blooms, in particular during spring (Eiler and Bertilsson 2004; Šimek et al. 2008). These bacteria harbor fast-growing genotypes that tend to respond quickly to substrate pulses (e.g., phytoplankton exudates; Zeder et al. 2009). For example, CF targeted by the newly designed probe Flu-736 were highly abundant during a spring phytoplankton bloom in Lake Zurich (E. Eckert, unpubl.).

However, in order to appreciate the significance of our results, one should also consider reversing the above argument: Much of our current knowledge about the seasonal changes of lacustrine (or coastal marine) microbial assemblages is derived from studies that have investigated a single depth layer at a single sampling location (Allgaier and Grossart 2006; Newton et al. 2006; Wu and Hahn 2006*a*). Although there are unquestioned logistic reasons for such a sampling strategy, it is equally obvious from our data that it might not always be appropriate to extrapolate the results from such time series across larger spatial scales. Therefore, additional longitudinal data from different times of the year would be needed to better understand

the spatial aspect of microbial dynamics over seasonal scales or in response to environmental perturbation. In sum, our study is a first baseline to understand the spatial niche differentiation of bacterioplankton populations in a stratified lake. Shifts in the microbial diversity usually result in changes in the processing of organic matter and different—even very closely related—microbes fulfill different ecological functions (e.g., different *Polynucleobacter* sp. [Hahn et al. 2011] or *Limnohabitans* sp. [Kasalicky et al. 2010]). The taxonomic resolution of our study is very high with the application of genus- to species-specific probes; therefore, our study provides an important step in understanding the role of microbes in the spatial structuring of nutrient cycling and ecosystem functioning.

Acknowledgments

We thank Eugen Loher and Stefan Neuenschwander for help during sampling and the water supply company Zurich for the provision of chemical data. The helpful suggestions and comments of two anonymous reviewers are highly appreciated. This study was supported by the Swiss National Science Foundation (SNF, Project 31003A-117765) and, as part of the European Collaborative Research Program (EUROCORES) Ecological and Evolutionary Functional Genomics (EuroEEFG) of the European Science Foundation (ESF), by the SNF Project 31EE30-132771.

References

Allgaier, M., and H. Grossart. 2006. Diversity and seasonal dynamics of *Actinobacteria* populations in four lakes in northeastern Germany. Appl. Environ. Microbiol. **72:** 3489–3497, doi:10.1128/AEM.72.5.3489-3497.2006

Amann, R., and B. M. Fuchs. 2008. Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques. Nat. Rev. Microbiol. **6:** 339–348, doi:10.1038/nrmicro1888

Atamna-Ismaeel, N., and others. 2008. Widespread distribution of proteorhodopsins in freshwater and brackish ecosystems. ISME J. **2:** 656–662, doi:10.1038/ismej.2008.27

Blackburn, N., T. Fenchel, and J. Mitchell. 1998. Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science **282:** 2254–2256, doi:10.1126/science.282.5397.2254

Blom, J. F., H. I. Baumann, G. A. Codd, and F. Jüttner. 2006. Sensitivity and adaptation of aquatic organisms to oscillapeptin J and [D-Asp3,Dhb7]microcystin-RR. Arch. Hydrobiol. **167:** 547–559, doi:10.1127/0003-9136/2006/0167-0547

Bossard, P., and others. 2001. Limnological description of the Lakes Zürich, Lucerne, and Cadagno. Aquat. Sci. **63:** 225–249, doi:10.1007/PL00001353

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol. Oceanogr. **47:** 453–470, doi:10.4319/lo.2002.47.2.0453

Comerma, A., J. C. Garcia, M. Romero, J. Armengol, and K. Šimek. 2003. Carbon flow dynamics in the pelagic community of the Sau Reservoir (Catalonia, NE Spain). Hydrobiologia **504:** 87–98, doi:10.1023/B:HYDR.0000008511.45153.aa

Dimitriu, P. A., H. C. Pinkart, B. M. Peyton, and M. R. Mormile. 2008. Spatial and temporal patterns in the microbial diversity of a meromictic soda lake in Washington State. Appl. Environ. Microbiol. **74:** 4877–4888, doi:10.1128/AEM.00455-08

Eiler, A., and S. Bertilsson. 2004. Comparison of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ. Microbiol. **6:** 1228–1243, doi:10.1111/j.1462-2920.2004.00657.x

Feuillade, M., J. Bohatier, G. Bourdier, P. Dufour, J. Feuillade, and H. Krupka. 1988. Amino-acid uptake by a natural population of *Oscillatoria rubescens* in relation to uptake by bacterioplankton. Arch. Hydrobiol. **113:** 345–358.

Fuchs, B., F. Glöckner, J. Wulf, and R. Amann. 2000. Unlabeled helper oligonucleotides increase the in situ accessibility to 16S rRNA of fluorescently labeled oligonucleotide probes. Appl. Environ. Microbiol. **66:** 3603–3607, doi:10.1128/AEM.66.8.3603-3607.2000

Gich, F., K. Schubert, A. Bruns, H. Hoffelner, and J. Overmann. 2005. Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. Appl. Environ. Microbiol. **71:** 5908–5919, doi:10.1128/AEM.71.10.5908-5919.2005

Giovannoni, S. J., and others. 2008. The small genome of an abundant coastal ocean methylotroph. Environ. Microbiol. **10:** 1771–1782, doi:10.1111/j.1462-2920.2008.01598.x

———, and ———. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science **309:** 1242–1245, doi:10.1126/science.1114057

Glöckner, F. O., E. Zaichikov, N. Belkova, L. Denissova, J. Pernthaler, A. Pernthaler, and R. Amann. 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. Appl. Environ. Microbiol. **66:** 5053–5065, doi:10.1128/AEM.66.11.5053-5065.2000

Grossart, H. P., F. Levold, M. Allgaier, M. Simon, and T. Brinkhoff. 2005. Marine diatom species harbour distinct bacterial communities. Environ. Microbiol. **7:** 860–873, doi:10.1111/j.1462-2920.2005.00759.x

Hahn, M. W., E. Lang, U. Brandt, and C. Sproer. 2011. *Polynucleobacter acidiphobus* sp. nov., a representative of an abundant group of planktonic freshwater bacteria. Int. J. Syst. Evol. Microbiol. **61:** 788–794, doi:10.1099/ijs.0.023929-0

Hoppe, H.-G., K. Gocke, R. Koppe, and C. Begler. 2002. Bacterial growth and primary production along a north–south transect of the Atlantic Ocean. Nature **416:** 168–171, doi:10.1038/416168a

Hornák, K., J. Jezbera, and K. Šimek. 2008. Effects of a *Microcystis aeruginosa* bloom and bacterivory on bacterial abundance and activity in a eutrophic reservoir. Aquat. Microb. Ecol. **52:** 107–117, doi:10.3354/ame01210

Jezberová, J., J. Jezbera, U. Brandt, E. S. Lindström, S. Langenheder, and M. W. Hahn. 2010. Ubiquity of *Polynucleobacter necessarius* ssp. *asymbioticus* in lentic freshwater habitats of a heterogenous 2000 km^2 area. Environ. Microbiol. **12:** 658–669, doi:10.1111/j.1462-2920.2009.02106.x

Johnson, Z. I., E. R. Zinser, A. Coe, N. P. Mcnulty, E. M. S. Woodward, and S. W. Chisholm. 2006. Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. Science **311:** 1737–1740, doi:10.1126/science.1118052

Kasalicky, V., J. Jezbera, K. Šimek, and M. W. Hahn. 2010. *Limnohabitans planktonicus* sp. nov., and *Limnohabitans parvus* sp. nov., two novel planktonic *Betaproteobacteria* isolated from a freshwater reservoir and emended description of the genus *Limnohabitans*. Int. J. Syst. Evol. Microbiol. **60:** 2710–2714, doi:10.1099/ijs.0.018952-0

Kirchman, D. L. 2002. The ecology of *Cytophaga–Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol. **39:** 91–100.

———, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2005. Biogeography of major bacterial groups in the Delaware estuary. Limnol. Oceanogr. **50:** 1697–1706, doi:10.4319/lo.2005.50.5.1697

Ludwig, W., and others. 2004. ARB: A software environment for sequence data. Nucleic Acids Res. **32:** 1363–1371, doi:10.1093/nar/gkh293

Newton, R. J., S. E. Jones, M. R. Helmus, and K. D. McMahon. 2007. Phylogenetic ecology of the freshwater *Actinobacteria* acI lineage. Appl. Environ. Microbiol. **73:** 7169–7176, doi:10.1128/AEM.00794-07

———, A. D. Kent, E. W. Triplett, and K. D. McMahon. 2006. Microbial community dynamics in a humic lake: Differential persistence of common freshwater phylotypes. Environ. Microbiol. **8:** 956–970, doi:10.1111/j.1462-2920.2005.00979.x

O'Sullivan, L. A., J. Rinna, G. Humphreys, A. J. Weightman, and J. C. Fry. 2005. *Fluviicola taffensis* gen. nov., sp. nov., a novel freshwater bacterium of the family *Cryomorphaceae* in the phylum '*Bacteroidetes*'. Int. J. Syst. Evol. Microbiol. **55:** 2189–2194, doi:10.1099/ijs.0.63736-0

Pernthaler, J. 2005. Predation on prokaryotes in the water column and its ecological implications. Nat. Rev. Microbiol. **3:** 537–546, doi:10.1038/nrmicro1180

Reichart, I., and M. Simon. 1996. Horizontal variability of bacterioplankton growth dynamics in a large lake. Aquat. Microb. Ecol. **11:** 31–41, doi:10.3354/ame011031

Rinke, K., A. M. R. Huber, S. Kempke, M. Eder, T. Wolf, W. N. Probst, and K. O. Rothhaupt. 2009. Lake-wide distributions of temperature, phytoplankton, zooplankton, and fish in the pelagic zone of a large lake. Limnol. Oceanogr. **54:** 1306–1322, doi:10.4319/lo.2009.54.4.1306

Salcher, M. M., J. Pernthaler, and T. Posch. 2010. Spatiotemporal distribution and activity patterns of bacteria from three phylogenetic groups in an oligomesotrophic lake. Limnol. Oceanogr. **55:** 846–856, doi:10.4319/lo.2009.55.2.0846

———, ———, and ———. 2011. Seasonal bloom dynamics and ecophysiology of the freshwater sister clade of SAR11 bacteria 'that rule the waves' (LD12). ISME J. **5:** 1242–1252, doi:10.1038/ismej.2011.8

———, ———, M. Zeder, R. Psenner, and T. Posch. 2008. Spatio-temporal niche separation of planktonic *Betaproteobacteria* in an oligo-mesotrophic lake. Environ. Microbiol. **10:** 2074–2086, doi:10.1111/j.1462-2920.2008.01628.x

Salka, I., V. Moulisova, M. Koblizek, G. Jost, K. Jurgens, and M. Labrenz. 2008. Abundance, depth distribution, and composition of aerobic bacteriochlorophyll a-producing bacteria in four basins of the central Baltic Sea. Appl. Environ. Microbiol. **74:** 4398–4404, doi:10.1128/AEM.02447-07

Schattenhofer, M., B. M. Fuchs, R. Amann, M. V. Zubkov, G. A. Tarran, and J. Pernthaler. 2009. Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. Environ. Microbiol. **11:** 2078–2093, doi:10.1111/j.1462-2920.2009.01929.x

Sharma, A. K., and others. 2009. Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater *Actinobacteria*. ISME J. **3:** 726–737, doi:10.1038/ismej.2009.13

Šimek, K., M. Comerma, J.-C. Garcia, J. Nedoma, R. Marce, and J. Armengol. 2011. The effect of river water circulation on the distribution and functioning of reservoir microbial communities as determined by a relative distance approach. Ecosystems **14:** 1–14.

———, K. Hornak, J. Jezbera, J. Nedoma, P. Znachor, J. Hejzlar, and J. Sed'a. 2008. Spatio-temporal patterns of bacterioplankton production and community composition related to phytoplankton composition and protistan bacterivory in a dam reservoir. Aquat. Microb. Ecol. **51:** 249–262, doi:10.3354/ame01193

———, V. Kasalicky, K. Hornak, M. W. Hahn, and M. G. Weinbauer. 2010. Assessing niche separation among coexisting *Limnohabitans* strains through interactions with a competitor, viruses, and a bacterivore. Appl. Environ. Microbiol. **76:** 1406–1416, doi:10.1128/AEM.02517-09

———, and others. 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Appl. Environ. Microbiol. **67:** 2723–2733, doi:10.1128/AEM.67.6.2723-2733.2001

Van Den Wyngaert, S., M. M. Salcher, J. Pernthaler, M. Zeder, and T. Posch. 2011. Quantitative dominance of seasonally persistent filamentous cyanobacteria (*Planktothrix rubescens*) in the microbial assemblages of a temperate lake. Limnol. Oceanogr. **56:** 97–109, doi:10.4319/lo.2011.56.1.0097

Winter, C., T. Hein, G. Kavka, R. L. Mach, and A. H. Farnleitner. 2007. Longitudinal changes in the bacterial community composition of the Danube River: A whole-river approach. Appl. Environ. Microbiol. **73:** 421–431, doi:10.1128/AEM.01849-06

Wu, Q., and M. Hahn. 2006*a*. Differences in structure and dynamics of *Polynucleobacter* communities in a temperate and a subtropical lake, revealed at three phylogenetic levels. FEMS Microbiol. Ecol. **57:** 67–79.

———, and ———. 2006*b*. High predictability of the seasonal dynamics of a species-like *Polynucleobacter* population in a freshwater lake. Environ. Microbiol. **8:** 1660–1666, doi:10.1111/j.1462-2920.2006.01049.x

Zeder, M., and J. Pernthaler. 2009. Multispot live-image autofocusing for high-throughput microscopy of fluorescently stained bacteria. Cytom. Part A **75A:** 781–788, doi:10.1002/cyto.a.20770

———, S. Peter, T. Shabarova, and J. Pernthaler. 2009. A small population of planktonic *Flavobacteria* with disproportionally high growth during the spring phytoplankton bloom in a prealpine lake. Environ. Microbiol. **11:** 2676–2686, doi:10.1111/j.1462-2920.2009.01994.x

Zwirglmaier, K., and others. 2008. Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ. Microbiol. **10:** 147–161.

Associate editor: Mikhail V. Zubkov

Received: 16 February 2011
Accepted: 18 July 2011
Amended: 12 July 2011